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Efficacy of prepartum intramammary lactating cow treatment in dairy heifers

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EFFICACY OF PREPARTUM INTRAMAMMARY LACTATING COW TREATMENT IN DAIRY HEIFERS

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ABSTRACT

Mastitis in prepartum dairy heifers has been recognized as a significant economic problem for the dairy industry. Intramammary infusion of cephapirin sodium was completed in 20 treatment animals and the results were compared to 25 non-treated control animals. Bacteriological data from the quarter milk samples were used to determine and compare initial infection rates and cure rates following calving between treatment and control groups. Comparisons were also made between groups for differences in reproductive performance, milk yield, somatic cell count and milk ketone concentration.

Milk yield did not differ between groups for either DHIA monthly average kg/day, 305 day actual milk yield or 305 day mature equivalent yield (305ME). The average weekly milk weights (kg/d) between groups were different ($P<.05$). Reproductive performance between groups was not significantly different. Milk ketone concentration did not differ at weeks 1 or 2. Somatic cell count score (SCS) at 200 days in milk were not different between groups, but the treatment group exhibited a trend towards lower cell counts ($P<.15$). SCS in the treatment group tended to be lower ($P<.15$) during the average of the first 3 DHIA test periods. Prepartum treatment of dairy heifers significantly improved cure rates in the treatment group ($P<.05$), moderately reduced SCC throughout lactation, and did not affect milk yield. While there were no significant differences between treatment groups in milk ketone concentrations, evidence suggests that infections caused by major mastitis pathogens may increase metabolic stress on primiparous heifers. Further research in the use of prepartum lactating cow antibiotics in heifers is needed.

CHAPTER 1. INTRODUCTION

Mastitis continues to be a major problem in the dairy industry resulting in lost income, shortened productive life, decreased animal quality of life, and decreased milk quality. Estimated financial losses for the industry approach $2 billion annually. Annual losses in states such as New York and Louisiana have been estimated at $150 million and $15 million, respectively (39, 47). Recent loss estimates total $184.40 per cow/year total (4). Reduced production accounts for more than half of the lost income, while discarded milk, animal replacement costs, extra labor, medication, and veterinary services are further contributors. Proven control measures based on scientific research have decreased the prevalence of mastitis in most herds. Only in the past 20 years has heifer mastitis been identified as a major cause of lost income and decreased animal performance. There are several reasons why heifer intramammary infections (IMI) should be of concern to the dairy industry: 1.) IMI in heifers adversely affects proper growth and proliferation of mammary secretory cells, affecting future production (81). 2.) Heifers may serve as new vectors of contagious mastitis pathogens in herds that otherwise practice good mastitis management (69). 3.) Heifers infected at the onset of lactation may be at higher risk for problems related to sub-clinical and clinical mastitis. These problems include decreased reproductive performance (2, 24, 72), increased likelihood of elevated somatic cell count (46), and increased risk of premature culling. As a result, the animal's genetic potential for milk production may never be reached. Research to date indicates that antibiotics may be a feasible way to control new IMI in heifers when prevention has failed. Mastitis in prepartum, primigravid heifers may be more likely cured or prevented, in comparison to mastitis in lactating cows (42, 52, 53). As dairy herds increase in size and pressure increases to reduce the national somatic cell count standard, mastitis control and prevention will become more important, especially that related to spread of contagious infections such as those caused by *Staphylococcus aureus*.

CHAPTER 2. LITERATURE REVIEW

2.1 Prevalence and Economics

Intramammary infection rates in heifers can be as high as 97% (46). *Staphylococcus aureus* is perhaps the best example of a mastitis pathogen present in heifers that continues to cause significant financial losses despite increased knowledge in the fields of dairy management, microbiology, pharmacology, and biotechnology. *Staphylococcus aureus* is considered a major contagious mastitis pathogen. It has been isolated from nulliparous, primigravid, primiparous, and multiparous animals. Prevalence of *S. aureus* in prepartum and primigravid heifers ranges from 2.6 to 37.1%, (15, 38, 65, 82, 83), with percentage of infected quarters ranging from 0.7 to 15.4% (50, 52, 56, 65, 73, 82). *Staphylococcus aureus* has also been found to account for 2.4 to 46.2% of all intramammary infections (IMI) in primiparous animals near parturition (38, 44, 46, 48, 82, 83). Prevalence of *S. aureus* in postpartum, primiparous heifers ranges from 2.8 to 20.4% (15, 38, 68), with the percentage of infected quarters ranging from 0.4 to 3.4 (34, 38, 49). Wilson et al., (90) and Fox et al. (12) found *S. aureus* prevalence in mixed parity animals to range from 9.1 to 33.7%. Using data from multiparous cows, Mathews et al. (34) found that 2.3% of all quarters were infected before calving and that 0.6% of quarters were infected with *S. aureus* postcalving. It is understandable that the percentage of *S. aureus*-infected animals and quarters varies, as *S. aureus* IMI have proven difficult to consistently culture and isolate. Estimates indicate that 75 to 91% of *S. aureus* IMI are found in the first attempt to culture, with 98% confirmed with three attempts (75, 76).

Staphylococcus aureus infections in breeding age heifers may occur as early as 9 months of age and persist for periods of 1 year or more, even into the first lactation. The consequences of *S. aureus* IMI are clear. These early infections may damage mammary development and impair

milk production for the animals' productive life (46, 81). Natzke et al. (39) found that 305-day milk yield decreased by 773 kg when one quarter was infected with *S. aureus*. Wilson et al. (91) estimated financial losses of $185.51 per case of *S. aureus* based on 305-day mature equivalent milk yield compared with uninfected herdmates. Jaenicke et al. (26) found that when heifers were infused with dry cow antibiotics prepartum, net revenue increased $174.92 due to increased milk production. Decreased reproductive performance (2, 72) may occur due to *S. aureus* IMI. Additionally, premature culling may be necessary to control *S. aureus*.

2.2 Modes of Transmission

Various mechanisms of transmission have been identified, including flies (21, 58) and fomites found in the milking parlor, such as milking equipment, milkers' hands, common udder cloths, and strip cups (11). Boddie et al. (3) found IMI prevalence of 5.2% in herds that practiced fly control vs. 55.2% in herds that did not. Milk droplet impacts on teat ends due to malfunctioning milking equipment may also induce new *S. aureus* IMI (4). Literature indicates that *S. aureus* can be isolated from the environment, mammary secretions, streak canals, and/or body sites of most dairy animals (33, 69, 70). Researchers have concluded that reservoirs for infection are most likely udders of infected heifers and cows (69). *Staphylococcus aureus* isolated and "fingerprinted" as those bacteria responsible for IMI were found to most likely originate from *S. aureus*-infected milk or body sites and could be transmitted from animal to animal by flies (21, 69). Additional means of transmission include calves suckling other calves, especially those fed waste or mastitic milk (41, 66). It is possible that all mechanisms of spread of *S. aureus* IMI are not yet known.

2.3 Pathology and Virulence Factors

In addition to being commonly isolated from dairy animals, *S. aureus* is highly pathogenic

and persistent in the mammary gland. Virulence factors possessed by *S. aureus* are partially responsible for the subclinical, chronic type of mastitis that causes damage to secretory cells of the mammary gland (17, 35, 80). *Staphylococcus aureus* IMI cannot be solely characterized as subclinical and chronic. In rare cases, *S. aureus* mastitis can be peracute, gangrenous, and fatal (77). Literature indicates that not all virulence factors relevant to *S. aureus* IMI have been discovered (78). Known virulence factors help *S. aureus* to evade the host's immune defense and resist infused or parenteral antibiotics. Hyaluronidase is an enzyme possessed by *S. aureus* that enables it to penetrate mammary tissue to which it has adhered. Microabcesses form and eventually develop scar tissue which is impermeable to antibiotics. *Staphylococcus aureus* can be released if the microabcesses or scar tissue breaks down. This contributes to chronicity, clinical flare-ups, and the ability of the infection to spread further within the gland. *Staphylococcus aureus* also possesses another enzyme, coagulase, which is used to differentiate *S. aureus* from other *Staphylococcus* species. Coagulase reacts with inflammation products, yielding fibrin-like clots. These clots inhibit leukocyte mobility and hinder the action of the host's immune system phagocytes. These clots may also prevent drainage of milk from ducts of the gland and lead to stasis or destruction of secretory cells (4).

Staphylococcus aureus also releases toxins, including alpha, beta, gamma, and delta toxins. Of these, alpha toxin appears to be the most toxic. It is particularly harmful to mammary tissues causing vasoconstriction, which leads to localized ischemia and cell necrosis (22). In times of rapid *S. aureus* growth, the effects of alpha toxin may lead to gangrenous mastitis (4). Additionally, Foster et al. (10) noticed a lack of macrophages and neutrophils in areas where alpha toxin-producing *S. aureus* were growing in *in vitro* mouse mastitis models. The authors theorized that this was due to decreased chemotaxis of macrophages and neutrophils into regions

where alpha toxin-positive bacteria were growing. Early research found that beta and gamma toxins were mostly tissue irritants, with beta toxin being the most predominant toxin of *S. aureus* isolated from animals (22). However, beta toxin has been found to increase bacterial growth in in vitro mouse mastitis experiments (10).

Capuco et al. (5) found that alpha and beta toxins and leukocidin caused cell damage and decreased secretory activity in mammary explants. More recent research indicates that alpha and beta toxins may play a significant role in *S. aureus* adherence to mammary epithelial cells. Some research has contradicted the idea that adherence is necessary for establishment of *S. aureus* IMI (1). Cifrian et al. (6) evaluated factors affecting *S. aureus* adherence to cultured mammary epithelial cells. Their data suggested that cellular damage by alpha toxin is a necessary step for *S. aureus* adherence in the mammary gland. However, the authors were unsure whether *S. aureus* adhered to cells damaged solely by the alpha toxin or to cells with exposed basement membrane and cellular matrix. Cifrian and Guidry (7) later found that *S. aureus* adherence to cultured mammary epithelial cell monolayers was reduced when both alpha and beta toxins were neutralized by antibodies to alpha and beta toxins. They felt that this information indicated that alpha and beta toxins could influence *S. aureus* virulence by increasing the ability of *S. aureus* to adhere to mammary tissue. This study (7) agrees with earlier results (6), in that damage caused by alpha and/or beta toxins is at least partially responsible for *S. aureus* adherence to mammary epithelial cells. In addition, the later data (7) suggested that the bacterial cell wall and not the capsule contained the adherence factors. Frost et al., (18) and Wanasinghe (86) studied the ability of *S. aureus* to adhere to epithelial cells and found that adherence to mammary epithelial cells improved *S. aureus* pathogenicity. Adherence is made possible by bacterial surface fibronectin binding protein which binds to fibronectin present on the surface of mammary

epithelial cells (43, 84). This binding is dependent on the number of binding sites (83). Froman et al. (17) and Wadstrom et al. (85) indicated that epithelial damage is required for *S. aureus* to adhere to and colonize tissue. In fact, when a vaccine against these fibronectin-binding proteins was used in lactating Jersey cattle, new IMI decreased by 35.8% (43). Leukocidin is another virulence factor that interferes with the mammary cell's immune system. Leukocidin interferes with phagocytosis by causing cytolysis of both polymorphonuclear leukocytes (PMN) and macrophages (14, 22).

Components of the cell wall of *S. aureus* can also contribute to virulence. The chief component, peptidoglycan, causes delayed hypersensitivity which can lead to flare-ups in chronic cases of subclinical *S. aureus* in which additional tissue damage results (22). A second component, teichoic acid, can be converted to teichuronic acid in vivo. The cell-mediated immune system (CMI) and humoral immune system may not recognize teichuronic acid after the conversion (22). Indeed, Sutra et al.(80) found that masking of teichoic acid was favored when *S. aureus* was grown on SA 110 agar or milk agar. They hypothesized that this may lead to increased resistance to phagocytosis by polymorphonuclear leukocytes (PMNs). Protein A is the third cell wall component that may contribute to *S. aureus* virulence by binding to the Fc portion of IgG. By doing this, Protein A prevents opsonization of *S. aureus* by IgG (14, 22). However, there are two subtypes of IgG, IgG_1 and IgG_2. Protein A binds strongly to IgG_2, but only weakly binds to IgG_1 (79). Ruminant neutrophils have Fc receptors for IgG_2 only (87), and IgG_2 concentration increases during clinical mastitis (63). Given the high specificity of Protein A for IgG_2, and the relatively low milk concentration of IgG_2, this may indicate a lesser role of Protein A in *S. aureus* virulence (22). However, Pankey et al. (64) found that a Protein A vaccine increased spontaneous cure rate and decreased somatic cell counts in cows indicating a

significant role of Protein A in *S. aureus* virulence. Some strains of *S. aureus* may also form capsules or pseudocapsules (slime layer) (79). These may cover cell wall antigens and inhibit opsonization by complement and antibodies to cell wall components (79, 89). In concurrence, Nickerson (43) indicated that the *S. aureus* pseudocapsule/slime layer was sufficient to impede antibody and complement attachment, which would block phagocytosis. In fact, when cows were immunized with a vaccine designed to promote opsonization of the *S. aureus* capsule, phagocytic activity improved (23). Last of the virulence factors are "superantigens", which are controversial in their existence in *S. aureus* IMI. It has been hypothesized that the alpha, beta, gamma, delta toxins and leukocidin may be superantigens. Production of superantigens may lead to symptoms of shock immunosuppression in the host (32). Superantigens strongly stimulate all T-cell subtypes in an assortment of species. Literature indicates that superantigens can have detrimental effects on the function of CMI, actually diluting the cell populations necessary for specific immunity (32). The best known example of a superantigen is the staphylococcal enterotoxin B (32). Additional research needs to be done to fully understand and isolate possible superantigens possessed by *S. aureus* that cause bovine IMI (14).

Staphylococcus aureus can continue to resist the effects of antibiotics and/or the immune system if the microabcesses and scar tissue present from an established infection are bypassed. Literature indicates that the presence of milk in the gland can lower the efficacy of infused antibiotics (55, 59) and the effectiveness of the host intramammary immune phagocytes (62, 80). Presence of β-lactamase enzyme and conversion to L-forms are two additional ways *S. aureus* can avoid lysis in the mammary environment. β-lactamase (penicillinase) is an enzyme found in some strains of *S. aureus* that causes hydrolysis of the β-lactam ring. The β-lactamase enzyme of *S. aureus* has shown variability from herd to herd. This may be due to cow individuality and

antibiotic treatment habits. In one study, resistance to penicillin by *S. aureus* ranged from 0 to 60% between herds, with an overall average of 7% resistance (60). Additionally, *S. aureus* can be induced to L-forms. It is thought that L-forms of *S. aureus* act as a transition stage to survive conditions such as disruption of bacterial cell wall synthesis by antibiotics that are deleterious to cellular integrity. Cell survival is possible due to lack of an organized cell wall in these *S. aureus* L-forms. L-forms provide *S. aureus* with benefits that include the ability to withstand antibiotic therapy, persist in the mammary gland, and re-emerge (flare-up) when conditions improve (54). Owens (54) noted that cows experimentally infected with 10^6 cfu vs. 10^3 cfu *S. aureus* had higher incidence of L-form *S. aureus* in mammary secretions. The author felt that the increased inflammation and subsequent increase in pH, electrolytes, and serum proteins present in the mammary secretions of cows infused with the higher concentration of *S. aureus* may have played an influential role in increasing in vivo L-form induction. This agrees with the work of Young and Dahlquist (92) who found that a high osmolality (5%) was optimal for induction, growth, and maintenance of L-forms of *S. aureus* in rabbits. It may be possible that an influx of proteins and ions, especially sodium and chloride, into the mammary gland during inflammation may provide conditions for L-form induction. Lastly, it appears that *S. aureus* can use phagocytes to protect itself from antibiotics. *Staphylococcus aureus* can be engulfed and withstand the lytic effects of PMNs (8,9). Furthurmore, Craven and Anderson (9) reasoned that antibiotics did not eradicate phagocytized intracellular *S. aureus* due to reduced bacterial growth rate within phagocytes, despite data showing that leukocyte permeability to some antibiotics may increase when *S. aureus* is engulfed by leukocytes. If the organisms are in a metabolically inactive or inert state, then antibiotics will have little or no effect on decreasing microbial cell wall integrity.

2.4 Control and Prevention

While *S. aureus* IMI may be difficult to eradicate, literature indicates it can be controlled. Proper milking procedure and hygiene may be the easiest and most economical way to control *S. aureus* IMI (25). Data indicates that teat and udder skin should be healthy before milking and free of sores, wounds, or chapping where *S. aureus* could colonize the teat end and surrounding skin (13). Cleanliness at milking time is also important. Minimal use of water and premilking teat antisepsis may reduce new *S. aureus* IMI (42). Additionally, the advent of postmilking teat antisepsis has been important in contributing to decreasing contagious IMI such as *S. aureus*. Natzke, et al (39) and Oliver and Mitchell (50) found that when teats were dipped after milking and cows were treated with penicillin-dihydrostreptomycin at dry-off, IMI caused by major mastitis pathogens decreased by 75% and 45%, respectively. Postdipping alone has been estimated to decrease the rate of new IMI by 50% (45). In another effort to decrease new *S. aureus* IMI, the effect of segregating *S. aureus*-infected animals and noninfected animals was tested (12, 91). Fox and Hancock (12) found no significant differences among herds that segregated *S. aureus*-infected cows and those that did not. Conversely, Wilson et al. (91) found that *S. aureus* prevalence decreased from 29.5 to 16.3%, while somatic cell count decreased from 600,000 cell/ml to 345,000 cells/ml in herds where *S. aureus* cows were segregated. Since *S. aureus* is a contagious mastitis pathogen that can be spread at milking, it appears that segregation is an acceptable form of control in herds where it is feasible and beneficial when other steps are taken simultaneously to control *S. aureus*. Additional ways to control and prevent *S. aureus* include the use of lactating and dry cow antibiotics in pregnant heifers and dry cows. Lactating cow therapy may be used to control *S. aureus* IMI. Lactating cow therapy alone may cure 30.4% of animals and 25% of quarters (41). Lactating cow therapy in conjunction with a *S. aureus*

bacterin was shown to decrease somatic cell count from 492,000 cells/ml to 84,000 cells/ml (74). Lactation cow therapy in conjunction with parenteral antibiotic treatment was found to cure 48% of animals and 51.4% of quarters (61). Dry cow therapy in adult cattle can be expected to prevent between 50 and 80% of new *S. aureus* IMI during the dry period (41). Literature indicates that dry cow treatment in primigravid heifers may reduce new *S. aureus* IMI by more than 90% (52, 56, 57, 83). Fortunately, heifer mastitis, when compared to lactating cow mastitis, may be more easily cured and/or prevented through the use of management practices, vaccines, and antibiotics.

Additionally, the advent of biotechnology may introduce new methods of controlling *S. aureus* IMI. (51). Perhaps the best current example is the work done by Kerr et al. (28) on the expression of the lysostaphin gene in mice. Lysostaphin has the ability to lyse the *Staphylococcus* spp. cell wall. In laboratory experiments, mice with the transgenic gene were experimentally infected with *S. aureus*. Mice with the gene were able to lactate, reproduce, and function normally, but more importantly, mice with high levels of expressed lysostaphin remained uninfected after 10^4 cfu/50 µl/gland *S. aureus* was inoculated via intramammary infusion. Similar studies have not been reported in the bovine. Biotechnology may be helpful in the control and prevention of *S. aureus* IMI, in addition to management practices, vaccines, and antibiotics.

2.5 Effect on Reproduction and Metabolic State

Heifer mastitis may have negative effects on metabolic state and reproductive performance of animals in early lactation (31). Research regarding the relation between ketone bodies and the immune system has been conflicting (16, 29). Research dealing with mastitis and ketone bodies has focused on coliform types of mastitis. Little work has been done on mastitis caused by gram-

positive pathogens (30, 31). From the limited research available, it appears that cows and heifers in negative energy balance are at increased risk for intramammary infection. Whether major or minor pathogens have a metabolic effect on heifers or cows in early lactation remains a largely untested hypothesis.

Reproductive performance in animals with clinical or sub-clinical mastitis is likely to be impaired (2, 24, 72). Little, if any, research exists on this topic with regards to primiparous heifers. It can be extrapolated from research examining reproduction in lactating animals that infections acquired by primiparous heifers in the prepartum period can have an effect on the subsequent lactation.

In conclusion, current knowledge implicates *S. aureus* as a major mastitis pathogen in heifers and lactating dairy animals. *Staphylococcus aureus* and *Staphylococcus* spp. compose a large portion of infections found in unbred and primigravid heifers. However, it appears that *Staphylococcus* spp. IMI have a significant percentage of spontaneous elimination in the mammary gland, when compared to *S. aureus*. Considering the information presented in this review, efforts focusing on preventing or treating IMI caused by *S. aureus* may be beneficial. For these reasons, we sought to evaluate the efficacy of administration of lactating cow antibiotics in heifers two weeks pre-partum. This project is being completed as part of the NE-1009 regional mastitis research mission. Universities across North America will be completing the project, using the same protocol, and the data will be pooled and presented as a group paper, thereby enhancing its value to the industry.

CHAPTER 3. MATERIALS AND METHODS

3.1 Animal Assignment and General Management

Forty six primigravid Jersey heifers from the Hill Farm Research Station dairy herd were randomly assigned to treatment or control groups. Eartags were assigned consecutively to heifers at birth. Animals with even numbered ear tags (n=21) were assigned to the treatment group while animals with odd numbered ear tags were assigned to the control group (n=25). Assignment based on eartag number was standard procedure in the NE-1009 multistate protocol that this trial was part of. There was no bias applied to the numbering of heifers related to milk production. Additionally, statistical analysis was performed and there were no differences in age of enrollment in the study between experimental groups (P=.90). Treatment heifers averaged 25.2 months of age with a range of 19-33 months, while control heifers averaged 25.3 months of age with a range of 21-32 months at calving. Overall, heifer numbers favored the odd numbered heifers (control). This was due to greater death losses and culling within heifers with even numbered ear tags prior to beginning this study. In addition, one even numbered heifer was dropped from the study due to the extremely long period of time (55 days) between first sampling and calving. This heifer was bred by natural service and no breeding date was available. Average time from antibiotic infusion to parturition was 16 days. Control animals received no infusion. The study was initiated in January 2002 and completed in April 2003. Heifers were housed on Bermuda grass pastures during the spring, summer and fall months, and had access to rye grass pastures during the winter months. All heifers received 2 kg of a commercial 16% crude protein dairy pellet daily. Heifers were bred by either artificial insemination (n=31) or natural service to an Angus bull (n=14). Approximately 14 days prior to calving, heifers were moved into the close up dry cow area. The dry cow area consisted of a 6

acre pasture containing 2 sheds bedded with sand. Shade was provided by trees. After moving to the dry cow area, heifers received 1 kg of a 16% dairy pellet and 1 kg of a 50% anionic salt/ 50% grist type grain mix, in addition to *ad libitum* bermuda grass hay. After parturition, heifers were milked twice daily in a double 2, side opening parlor. Lactating heifers spent the first week of lactation in a transition pasture where they received a total mixed ration (TMR) and had access to bermuda grass pasture for 3 hours. Cows were fed twice daily at approximately 0900 hr and 1500 hr. Refused TMR was removed from feedbunks each day prior to the morning feeding. The TMR consisted of 10 lbs of alfalfa hay, 5 lbs of whole cottonseed, 10 lbs of coarse ground corn, .25 lbs of sodium bicarbonate, .10 lbs of magnesium oxide, .25 lbs of soybean meal, and .25 lbs of a mineral premix. Following a negative test for antibiotic residue using a Delvotest P MINI kit (DSM Food Specialties, Menomomee Falls, WI), heifers were moved into the lactating herd where they were housed in a freestall barn bedded with kiln dried sawdust. Bedding was cleaned twice daily and replaced weekly. Heifers received approximately 3-8 hrs of pasture time outside of the freestalls each day. Pasture consisted of Bermuda grass in the summer months (May thru October) and annual ryegrass in the winter months (November-April).

3.2 Treatment and Heifer Mammary Secretion Sampling

Mammary secretions from individual, functional quarters were aseptically collected in duplicate at 14 days prior to expected date of calving (C-14), at calving (C+0) and at 7 (C+7), 14 (C+14) and 21 days (C+21) postpartum for microbiologic evaluation. An aliquot of the milk samples taken at C+7 and C+14 were saved and frozen for analysis of milk ketones. Teat canals of quarters which yielded insufficient secretion for microbiological testing at C-14 were swabbed as described by Trinidad, et al. 1990 (82). Additionally, composite samples were collected at

milkings 3, 6 and 10 postpartum for antibiotic residue testing. All samples other than teat canal swabs were stored frozen at -20º C for 24 hours or more prior to microbiologic evaluation. Teat canal swabs were plated immediately on blood agar. Quarter sampling procedure at all times was as follows: Teat ends were scrubbed for 15-30 seconds with cotton balls soaked in 70% isopropyl alcohol. The keratin plug was removed from the streak canal via manual manipulation. Mammary secretions were collected in 17 x 100 mm sterile polystyrene tubes with caps, until three quarters full. Composite samples were taken without aseptic technique. Debris was removed from each teat and 5 squirts of milk was hand stripped into a 15 ml plastic sample tube. Following aseptic sampling, heifers with even numbered ear tags had commercially available intramammary antibiotic (200mg cephapirin sodium, Fort Dodge Animal Health, Fort Dodge, IA) infused into each functional quarter. Heifers with odd numbered ear tags received no intramammary infusion after sampling. Teats of all heifers were immediately dipped using an iodine based barrier type teat dip following sampling or infusion.

3.3 Microbiological Evaluation of Secretion

Samples of mammary secretions from each quarter (.01 ml) were plated on corresponding quarters of 5% bovine blood trypticase soy agar plates containing .01% esculin. Plates were incubated at 37°C for 48 hours. Colony growth was presumptively identified as either *Staphylococcus* spp., *Streptococcus* spp. or coliform according to colony morphology, hemolytic characteristics, Gram stain and catalase test. Bacteria presumptively identified as staphylococci were transferred from the original sample plate to an isolation blood agar plate. Following 24 hours of incubation, the isolation plate colonies were used to perform coagulase tests and were identified using API Staph kits (bioMérieux Vitek Inc., USA, 595 Anglum Rd, Hazelwood, MO). Bacteria confirmed as streptococci using the catalase test were further classified as Group

B, C or other using Streptex streptococcal grouping kit (remel USA, Lenexa, KS). Bacteria exhibiting a gram negative reaction to Gram staining were further identified to the species level using a API 20E kit (bioMérieux Vitek, Inc., USA, Hazelwood, MO). All isolates identified to the species level were placed in 4 ml trypticase soy broth containing 10% glycerin and stored at 20°C for future reference.

A quarter was considered infected if the organism identified was found in duplicate samples taken on day C-14. Quarters identified as being infected on C-14 were defined as being cured if all postpartum isolates were negative with respect to the prepartum isolate. Cures in quarters of control heifers were considered to be spontaneous, while cures in treatment heifers were considered to result from antibiotic treatment at C-14.

3.4 Somatic Cell Count

Samples were taken at days C-14 and C+14 to compare differences between treatment and control groups in somatic cell count (SCC). Additionally, SCC values determined at monthly Dairy Herd Improvement Association (DHIA) tests were used for each animals first 10 months of lactation. Quarter samples were taken using aseptic technique and frozen for 24 hours at -20ºC. To run SCC, samples were thawed, and warmed to 33-37ºC in a water bath. No preservative was added to the C-14 and C+14 samples, while preservative (Bromopol, D&F Control Systems, Inc., Dublin, CA) was used for samples taken for DHIA SCC. Somatic cell count was determined using a Fossomatic electronic cell counter (ALSN Foss, Hillerød, Denmark). Mammary secretions that were too viscous to pipette were diluted 1:10 with sterile saline as described by Trinidad, et al. (81). All SCC were converted to somatic cell count score (SCS, logarithmic) using the statistics program SAS 6.12 for windows.

3.5 Milk Ketone Testing

Composite milk samples from C+7 and C+14 were used to measure levels of ketone bodies in milk from both control and treatment heifers. The Ketotest^{mc} ketone test strips used were imported by Elanco Animal Health and provided courtesy of Dr. Ken Leslie, University of Guelph. If β-hydroxybutyric acid was present in the milk samples, it passed through the reagent pad of the test strip and was converted by the enzyme β-hydroxybutyric dehydrogenase to acetoacetic acid. Color change of the reagent pad was due to conversion of NAD to NADH, which reduced nitotetrazolium blue to formazan. Formazan caused the purple color of a positive reagent pad for the milk ketone body β-hydroxybutyric acid. The higher the concentration of β-hydroxybutyric acid, the deeper the purple color observed on the reagent pad. Composite milk samples were frozen at -20ºC for at least 24 hours prior to testing. Milk samples were allowed to thaw to room temperature prior to time of testing. Samples were shaken gently, and the milk ketone reagent strips were dipped into the milk sample for 3 seconds. The strips were removed from the milk and shaken twice to remove excess milk. The strips were then placed on a countertop and observed for color change 1 minute after removal from milk. Color development was compared to a standardized color chart and recorded as 0, 50, 100, 200, 500, or 1000μmol/L.

3.6 Statistical Analysis

Differences in mean values were evaluated between control and treatment groups for milk yield at 200 days in milk (DIM), 305 day actual milk yield, 305 day mature equivalent (ME) milk yield, and the first 3 months of DHIA recorded milk yield and SCC. Additionally, differences in milk ketones and SCC were evaluated between heifers with at least one *S. aureus* infected quarter and those without a *S. aureus* infected quarter. Differences in somatic cell count (SCC) were evaluated between control and treatment heifers at days C-14 and C+14. Analysis of

mean values between groups for ketone levels at weeks 1 and 2, and ketone levels between heifers with at least one *S. aureus* infected quarter and heifers without a *S. aureus* infected quarter were completed. Seasonal effect on milk ketone levels underwent preliminary analysis but results were discarded because of insufficient numbers resulting from poor distribution of animals throughout each season of the experiment. Also, differences between control and treatment groups were evaluated for services per conception, days open, days to first breeding, and first service conception rate. Finally, differences in quarter cure rates between groups were analyzed.

Statistical evaluation was done using SAS version 6.12. The general linear model procedure (PROC GLM) was used, and care was taken to account for the unbalanced sample population. Statistical significance was declared at $P \leq 0.05$, while trends were declared at $P \leq 0.15$.

CHAPTER 4. RESULTS

4.1 Cure Rates

Cure rates were determined for the quarters of 20 (quarters= 80) treatment heifers and 25 (quarters= 99) control animals. Twenty one of twenty five heifers (84%) in the control group were infected in at least one quarter at 14 days before calving. In the treatment group, 17 of 20 heifers (85%) were infected in at least one quarter 14 days prior to parturition (Table 4.1). During the sampling period, one quarter was lost in the treatment group due to a fungal infection, and one quarter was lost in the treatment group as a result of gangrenous mastitis. One quarter in the control group was nonfunctional at the beginning of the sampling period and was never enrolled in the study.

Table 4.1. Percentage of infected heifers and quarters in control and treatment groups at 14 days prepartum (C-14)

	# Heifers			# Quarters		
	Infected	Uninfected	% infected	Infected	Uninfected	% infected
Control	21	4	84	59[a]	39	60
Treatment	17	3	85	41	39	51
Total	38	7	84	100	78	56

[a]One quarter was not counted due to sample contamination

As expected, treatment group quarters in treated heifers exhibited cure rates that were significantly improved compared to control quarters (P<.003). As shown in Table 4.2, intramammary infections in control quarters decreased by 21% while infections in treated quarters decreased 78%. Quarters with *S. aureus* infections decreased 23% in control heifers while treated heifers had an 80% decrease. Furthermore, results suggested that control heifers were more likely to acquire new infections during the sampling period (P<.09). Data for cure

Table 4.2. Number of infected quarters categorized by pathogen in control and treatment heifers

		# of quarters		
Pathogen	Group (T or C)	14 days prepartum C - 14	21 days postpartum C + 21(*)	% Reduction
S. aureus (SA)	C	13	8(2)	23
	T	10	2	80
CNS	C	33	24(1)	24
	T	24	5	79
Strep. spp.	C	7	1(6)	0
	T	2	0(1)	50
Mixed SA	C	3	1	66.7
	T	1	0	100
Mixed Non-SA	C	1	0	100
	T	4	0	100
Other	C	2	2	0
	T	0	0	—
Total =	C	58	37(9)	% 21
	T	37	7(1)	% 78

* Numbers in parentheses indicate quarters that were cured due to postpartum treatment. These quarters are not used in calculation of % reduction

CNS= Coagulase negative *Staphylococcus* spp.
SA= Mixed culture containing *Staphylococcus aureus*
Non- SA= Mixed culture containing no *Staphylococcus aureus* colonies

Table 4.3. Prevalence of mastitis pathogen isolation in heifers during late gestation and early lactation in the control group.

		# Control Quarters				
		C - 14	C + 0	C + 7	C + 14	C + 21
Uninfected		39	34	63	60	61
Staph. aureus		13	15	6	5	8
CNS		33	34	23	25	24
Streptococci		7	10	2	5	2
Mixed	*S. aureus*	3	1	__	1	1
	Non *S. aureus*	1	3	1	__	__
Other		2	2	3	2	2
Blind		1	1	2	2	2
Contaminated		1	__	__	__	__
Total =		100	100	100	100	100

CNS= Coagulase negative *Staphylococcus* spp.

Table 4.4. Prevalence of mastitis pathogen isolation in heifers during late gestation and early lactation in the cephapirin treatment group.

		# Treatment Quarters				
		C – 14[a]	C + 0	C + 7	C + 14	C + 21
Uninfected		39	66	68	70	72
Staph. aureus		10	5	3	3	2
CNS		24	5	4	5	5
Streptococci		2	2	1	—	—
Mixed	*S. aureus*	1	1	1	—	—
	Non *S. aureus*	4	—	—	1	—
Other		—	1	3	1	—
Blind		—	—	—	—	1
Contaminated		—	—	—	—	—
Total =		80	80	80	80	80

[a]Samples obtained before cephapirin infusion

CNS= Coagulase negative *Staphylococcus* spp.

Table 4.5. Number of quarters treated for clinical mastitis during the experimental period.

	Control			Treatment		
Pathogen isolated	Treated	Cured	% Cured	Treated	Cured	% Cured
Staph. aureus	3	2	66	2	0	0
CNS	1	1	100	---	---	---
Streptococci	7	6	86	1	1	100
Other	1	0*	---	1	0*	---
Total	**12**	**9**	**75**	**4**	**1**	**25**

*indicates quarter was rendered non-functional.

CNS= Coagulase negative *Staphylococcus* spp.

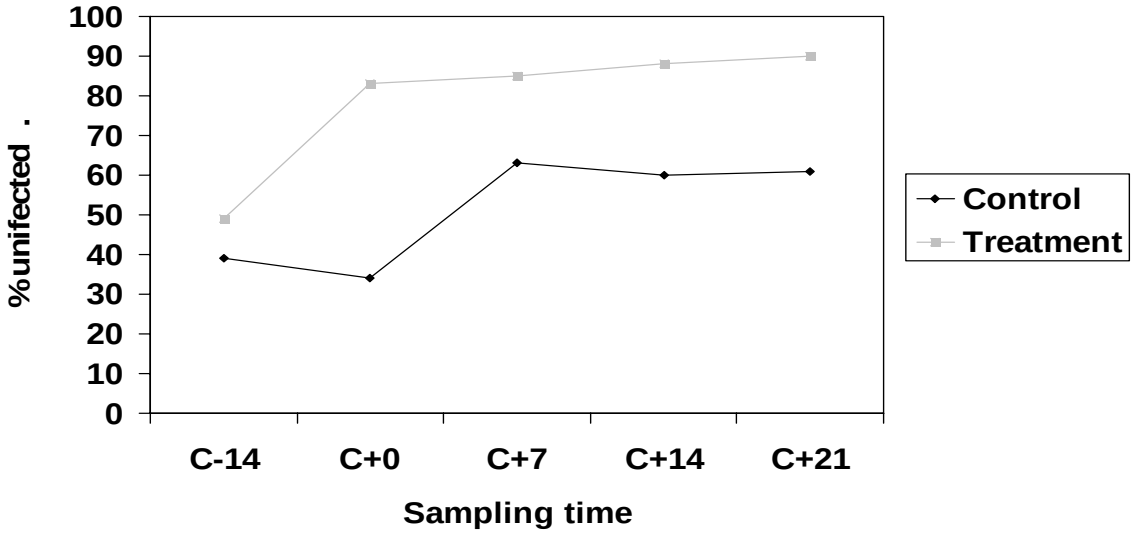

Figure 4.1: Percentage of uninfected quarters in control and treatment groups throughout sampling period

rates for major and minor pathogens isolated can be seen in Tables 4.2-4.5, with a summary shown in Figure 4.1.

Clinical mastitis during lactation was a problem during the sampling period of this study (Table 4.5). Twelve quarters in the control group (12%) were treated for clinical mastitis while four quarters (6.25%) in the treatment group were treated. Following treatment in the control group, 9 out of 12 quarters were cured (75%). Treatment of clinical quarters in the treatment group resulted in 1 out of 4 quarters being cured (25%). Quarters infected with *S. aureus* had cure rates of 66% and 0% for the control and treatment groups, respectively.

4.2 Milk Production

There were some significant differences in milk production/lactation between groups. Milk yield data is presented in Table 4.6 and Figure 4.2, and persistency data in Table 4.7. At 200 days in milk, the control group averaged 5.8%, or 1.1 kg/day, more milk than their treatment group herdmates. When comparing 305 day milk yield between groups, the control group produced 4.4%, or 242 kg, more milk than the treatment group. When average of the first 3 DHIA milk records was compared between groups, control animals averaged 5% more, or 1 kg/day, more milk in this early lactation period. One control animal calved with an intramammary fungal infection. This quarter was rendered nonfunctional with infusion of 60cc 2% Nolvasan. This heifer was milked the entire first lactation with the three remaining quarters. Additionally, one treatment animal calved with a gangrenous right rear quarter. The organisms isolated from this quarter were *Escherichia coli*, *Bacillus* spp., and *Streptococcus uberis*. The animal successfully completed her first lactation on the three remaining quarters. Further into lactation, 2 treatment heifers had quarters that atrophied and became non-functional. These quarters were previously infected with *Staphylococcus hyicus* and *Staphyloccous aureus*, and did not resolve following the prepartum intramammary treatment. The heifers completed their

Table 4.6. Comparison of milk yields of control and treatment heifers at different periods of lactation

	n	Control	SE(±)	n	Treatment	SE(±)	*P*
DHIA 3 month (100 day) average	25	19.6 kg	3.9	20	18.6 kg	4.2	.28
200 d daily lactation average	23	19.1 kg	0.61	20	18.0 kg	0.64	.24
305 d actual milk	23	5555 kg	1158	18[1]	5312 kg	1252	.40
305 d mature equivalent milk	23	7234 kg	1508	18[1]	7146 kg	1684	.83
Persistency, %	23	108.5	22.6	19	110.8	25.4	.57

[1] Two heifers from treatment group were eliminated between days 200 and 305 of lactation, but lactated enough for 200 day data and one of these lactated long enough for persistency data.

Table 4.7: Persistency[1] of milk production in heifers calving in warm weather vs. cooler weather.

	n	Control	SE(±)	n	Treatment	SE(±)	*P*
Warm[2]	8	101.9	36.0	10	106.5	33.7	.46
Cool[3]	15	114.5	29.6	9	115.9	38.6	.86
Average Persistency	23	108.5	22.6	19	110.8	24.4	.57
P (Warm vs. Cool)	-	.05		-	.13		

[1] Persistency is a DHIA defined term which measures the 30 day change in Fat Corrected Milk
[2] Warm weather = May 1 - Oct. 15.
[3] Cool Weather = Oct. 16 - April 31.

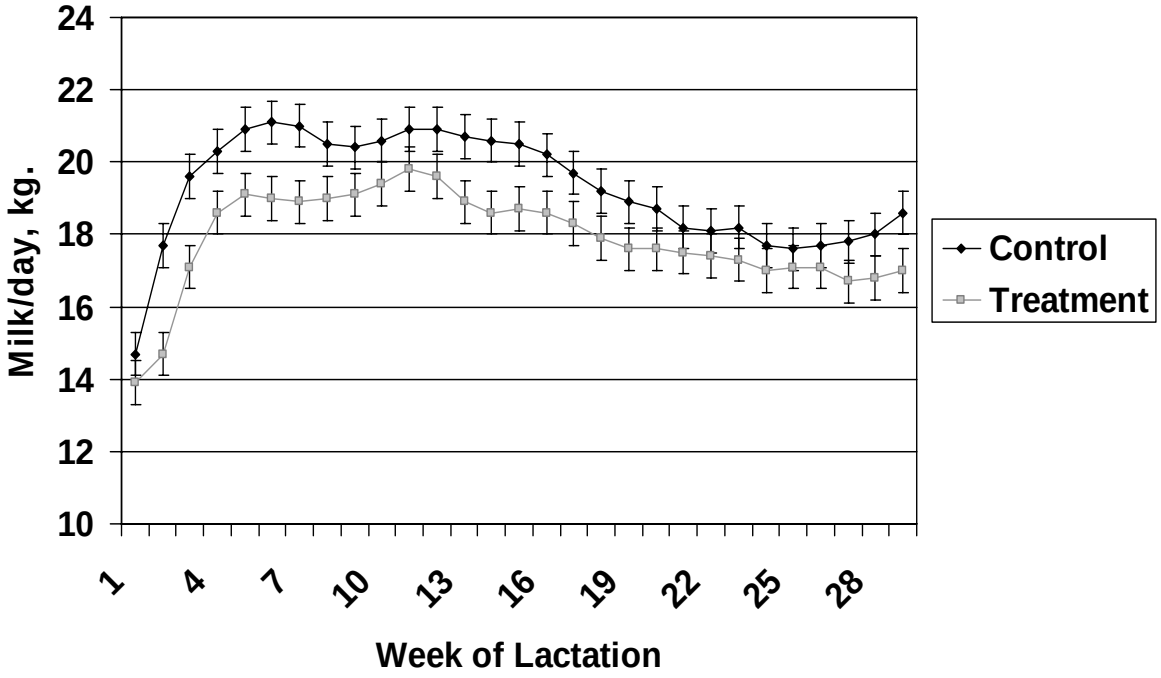

Figure 4.2: Comparison of average weekly milk weights: Control vs. treatment when milk yield was analyzed using split plot in time mixed model with covariate adjusted for calving date. Milk yield was significantly different (P=.0001).

lactation with the remaining 3 quarters. A summary of weekly production averages can be seen in Figure 4.2. The average weekly milk weights were significantly different (P=.0001)

4.3 Somatic Cell Counts

Prepartum antibiotic therapy appeared to lower somatic cell counts in treated heifers (Figures 4.3-4.5). This agrees with previous work done using both dry cow antibiotics and lactating cow antibiotics (53, 57, 83). Somatic cell counts between experimental groups were measured for each quarter at C-14 and C+14, and then measured again using DHIA composite averages for the first 3 test months and the first 200 days in milk (DIM). Complete data can be seen in Tables 4.8 and 4.9. Although there were no significant differences between groups, SCS was lower in control on C-14, and higher on C+14. There was a trend (P=.13) indicating control group quarters had differences at C-14. As shown in Table 8, control quarters had lower somatic cell score (SCS) at the beginning of the trial compared to treatment quarters. Yet, by day C+14 control quarters had decreased 48%, whereas the treatment quarters decreased 62%. This difference in SCS at C+14 was significant (P=.005), as shown in Table 4.9. This data indicates that prepartum antibiotic therapy is effective in lowering cell counts in heifers. Cell counts for the first three months of lactation, using DHIA records, were not different (Table 4.9). However, there was a tendency for treatment heifers to have lower average cell counts than control heifers throughout the 3 months (P=.07). When each month was compared individually between experimental groups, there were no differences. At the second and third months there were no differences between experimental groups. When cell counts were evaluated between experimental groups for the first 200 DIM, there were no differences but a trend was apparent (P=.11). From these results it appears that prepartum treatment was effective in reducing somatic cell count in heifers at parturition. Some of the effects of the treatment may persist throughout lactation, contributing to lower cell counts that extended into mid and late lactation.

Table 4.8. Comparison of individual quarter somatic cell score (SCS) between experimental groups at 14 days prepartum and 14 days postpartum.

	14 days prepartum (C-14)				14 days postpartum (C+14)			
	RFQ	LFQ	LRQ	RRQ	RFQ	LFQ	LRQ	RRQ
Control	7.6	7.8	7.5	7.7	4.4	3.5	3.5	4.4
Treatment	8.2	8.3	8.1	7.8	3.1	3.1	2.7	3.4

Table 4.9. Somatic cell count score (SCS): Control vs. treatment

	n	Control	SE(±)	n	Treatment	SE(±)	*P*
[1]C - 14	94	7.63	.79	79	8.11	.91	.13
[1]C +14	94	3.96	.41	79	3.07	.35	.005
Month 1	25	4.16	.83	20	3.18	.71	.11
Month 2	23	2.92	.61	20	2.43	.54	.47
Month 3	23	3.33	.69	20	2.86	.64	.46
[2]Mon 1-3 avg	25	4.15	.83	20	3.14	.70	.07
[2]200 D avg.	25	4.12	.82	20	3.29	.74	.11

[1] Samples at C - 14 and C + 14 were by individual quarter, while all others were composite.

[2] Data was averaged arithmetically ((M1+M2+M3)/3) and entered into SAS as an average, while data for months 1-3 was entered as an independent number to be evaluated and compared by SAS ((M1+M1+M1….)/n) as an individual month, thus the difference in SCS and P value.

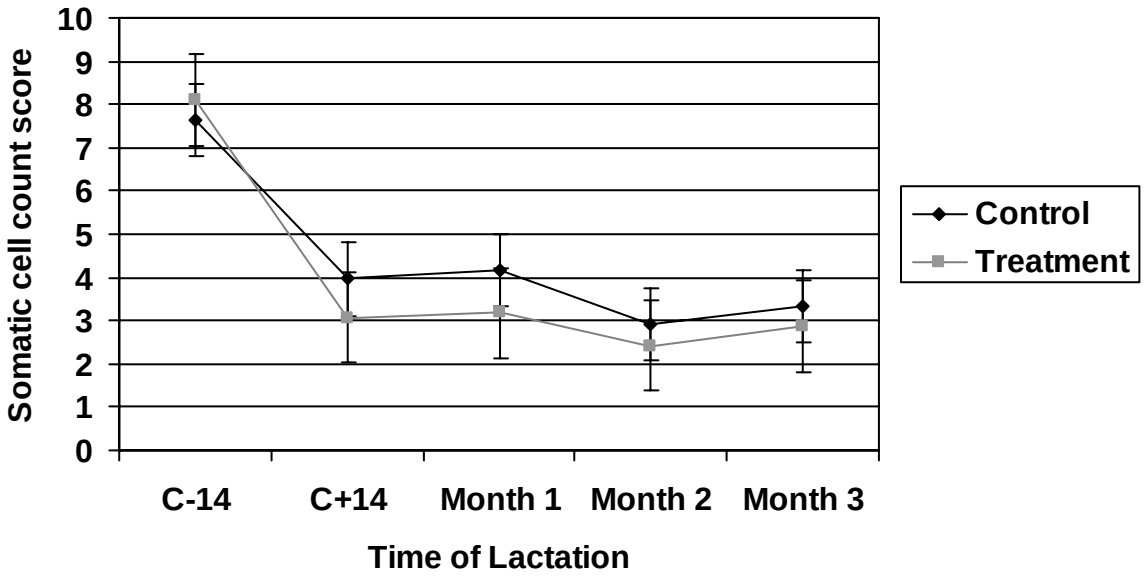

Figure 4.3: Somatic cell score at time of sampling: Control vs. Treatment

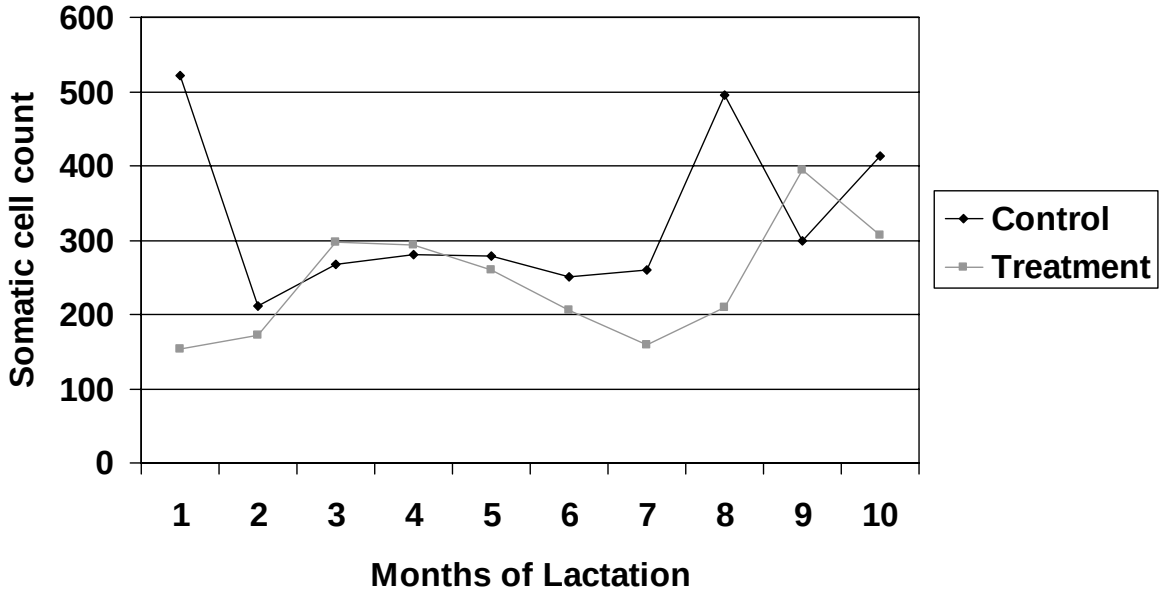

Figure 4.4: Somatic cell count for the first 10 months of lactation: Control vs. treatment

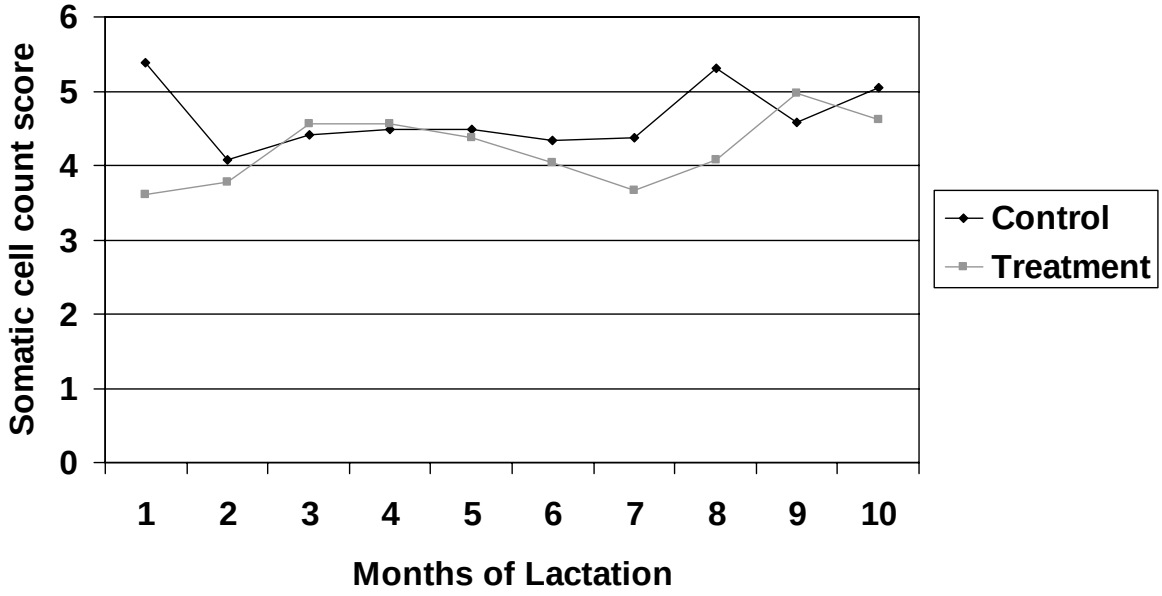

Figure 4.5: Somatic cell score for first 10 months of lactation: Control vs. Treatment

4.4 Reproduction

Data was evaluated for differences between services per conception, days open, days to first breeding, and first service conception rate as shown in Table 10 and 11. Reproductive data for one heifer in the treatment group was dropped from analysis due to an abortion at approximately 4 months. She remained in the herd and completed her lactation. Treatment animals had a mean of 2.74 services per conception; the control animals had a mean of 3.71 services per conception. Despite the numerical difference, there was no significant difference in services per conception between experimental groups (P=.33). Additionally, there were no differences in services per conception (P=.29) when animals with at least one *S. aureus* infected quarter were compared to animals without a *S. aureus* infection. However, heifers without *S. aureus* infection required 3.57 services per conception compared to 2.62 services per conception in heifers without a *S. aureus* infection. This is opposite to what would have been expected.

Days to first breeding were similar (65 vs. 64.4) between groups. Furthermore, the difference in days to first breeding between heifers with at least one *S. aureus* infected quarter, and those without a *S. aureus* infection were also similar (64.5 vs. 64.7). Days open between experimental groups and days open for heifers with or without *S. aureus* infections were not different. However, heifers without a *S. aureus* infection were open for 33 days longer than heifers with a *S. aureus* infection. Comparatively, days open between control and treatment groups were close (127.4 vs. 131.9). Finally, first service pregnancy rates did not differ between experimental groups (P=.81), or between *S. aureus* infection groups (P=.92). 31.6% of treatment heifers were pregnant to the first breeding while 29.2% of control heifers were pregnant to first breeding. All data analyzed for first service

Table 4.10: Reproductive data: Control vs. treatment.

	n	Control	SE(±)	n	Treatment	SE(±)	*P*
Services per conception	24	3.71	.76	19	2.74	.63	.33
Days open	23[1]	127.4	28.2	19	131.9	29.7	.89
Days to first breeding	24	65	14.9	19	64.4	13.1	.72
First service conception rate	24	1.72	.344	19	1.68	.386	.81

[1]One cow left the herd and never completed lactation, but was bred once.

Table 4.11: Reproductive data for heifers with *Staphylococcus aureus* infections vs. without *Staphylococcus aureus*.

	n	SA+	SE(±)	n	SA-	SE(±)	*P*
Services per conception	13	2.62	.73	30	3.57	.65	.29
Days open	12[1]	113.3	32.7	30	146.0	26.7	.30
Days to first breeding	13	64.5	17.9	30	64.7	11.8	.96
First service conception rate	13	1.69	.47	30	1.71	.307	.92

[1]One *Staphylococcus aureus* heifer was bred once, but left early in lactation.

conception rate was similar, regardless of infection status or treatment status.

4.5 Ketones

Milk ketone concentrations were measured at weeks 1 and 2 postpartum. Additionally, an investigation was made into the effects of *S. aureus* intramammary infection on ketone levels at both times. When week 1 ketones were compared between experimental groups, there were no differences (P=.32). When the ketone levels of heifers infected with *S. aureus* were compared to heifers without *S. aureus* infections, there were no significant differences (P=.96). There were no differences when milk ketone levels of heifers with *S. aureus* infections at week 2 were compared to those heifers without *S. aureus* infections (P=.31). A more detailed comparison found that there was evidence (P=.09) of a difference in ketone levels at week 2, when comparing control heifers with and without *S. aureus* infections. Data is shown in Tables 4.12-4.13.

Table 4.12: Milk ketones (µmol/L) in control and treatment groups at weeks 1 and 2.

	n	Control	SE(±)	n	Treatment	SE(±)	*P*
Week 1	22	63.6	13.6	18	120	28.3	.32
Week 2	22	201.4	42.9	18	166.7	39.3	.73

Table 4.13: Milk ketone concentration (µmol/L) in heifers with and without *Staphylococcus aureus* intramammary infection

	n	SA	SE(±)	n	Non SA	SE(±)	*P*
Week 1	9	93.1	31.0	31	90.6	16.3	.96
Week 2	12	232.2	67.1	28	135.8	25.7	.31

CHAPTER 5. DISCUSSION

5.1 Cure Rates

Overall, infected quarters in the treatment group had higher cure rates than infected quarters in the control group, despite more infections present in control quarters at the onset of sampling. This reinforces results of earlier studies indicating high efficacy of lactating cow intramammary antibiotics in heifers (52). In this study, infection rate in control quarters dropped 21%, while infection rate in treated quarters decreased by 78%. Overall infection rate of quarters with *S. aureus* decreased 23% in control quarters while decreasing 80% in treated quarters (Table 4.2). These numbers are remarkably similar to results of a 1992 study by Oliver et al.(52).

The predominate bacterial species isolated were *Staphylococcus* spp.. Previous research has shown that CNS are the most common in heifers regardless of location (52, 65, 82, 83). *Staphylococcus aureus* comprised 23% of all infections at day C-14. While this is greater than previously reported at other locations, (46, 49, 65), this percentage is consistent with previous data generated at this laboratory (82).

The type of pathogen, time of infection and timing of the antibiotic infusion of the gland may affect efficacy (53). One objective of this study was to sample all animals 14 days before calving. The average time of antibiotic administration in this study was 16 days before calving. It is possible that concentrations of antibiotics could decrease to non-therapeutic levels if the drug was administered too many days prior to calving. The time between decreased levels of antibiotic and calving may provide a window of opportunity for new IMI to occur, especially given the immunosuppression inherent in periparturient dairy cows. From C-14 to C+0, the control group acquired 2 new *S. aureus*, 1 new CNS, and 3

new *Streptococcus* spp. intramammary infections, while the treatment group acquired only 1 new coliform infection during the same time period. This indicates that infused antibiotics not only have an effect on cure rates for pre-existing infections, they also have a protective effect against new infections acquired in the periparturient period.

5.2 Milk Production

Past research has shown a significant increase in milk production of heifers treated with intramammary antibiotics prepartum (52, 53, 57). These researchers found Jersey heifers treated with antibiotics (n=114) at 7 and 14 days prepartum produced 531 kg more milk per lactation than control heifers (n=83). The increased milk production is commonly thought to occur from eradicating infections within the mammary gland and preventing damage to the secretory cells. However, the control group produced significantly more milk treatment groups in our study (Figure 4.2). This is in contradiction to previous studies examining milk production in heifers treated prepartum (53). In our trial, lower infection rates in the control group before calving may have had an effect on milk production. The control group had SCS that was 6% lower than the treatment group at C-14 (Table 4.9). This lower SCS would indicate a lower initial infection rate in the control group, which may have contributed to higher milk production by control heifers throughout the lactation (Figure 2).

An additional contributing factor may have been genetics. Efforts were made to examine genetic differences in Predicted Transmitting Ability (PTA) milk between the groups, but the number of animals unregistered or with unknown sires made this impossible. In a small research population, it would require very few genetically superior heifers to skew the data towards one group or another. Future studies may want to pay special attention to the number and genetics of trial animals. Additionally, the seasonal effect of calving,

warmer weather vs. cooler weather may have altered the data. It is well documented that lactating dairy cows are sensitive to heat stress. As ambient temperature and humidity increase, dry matter intake, milk production, and estrous activity decrease (88). In our study, more heifers in the treatment group calved during hot weather than in the control group. Of the 20 animals in the treatment group, 6 (30%) calved in mild weather (October 15 thru May 1). Of the 25 animals in the control group, 10 (40%) calved in mild weather. These differences could have been magnified by the small population of the experimental groups. In mild weather, dairy heifers would be expected to have higher dry matter intake, less fly stress, less heat stress and less overall metabolic stress. It would be expected that production would be slightly higher in heifers calving in mild weather, than in those calving in July, August, and September, as did the remainder of the treatment heifers. Table 5.1 shows the breakdown of heifers calving by season, as defined by the western calendar. It could be extrapolated that heifers calving during the cooler months and those that continued lactation during cooler months would be expected to have higher persistency and milk yield (19, 36). Persistency in milk production did not differ between treatment groups but did differ between warm or cold weather calving heifers in this trial (Table 4.9). Previous research has shown that environmental factors have an effect on milk yield and persistency in lactating sheep (19, 71). The low number of experimental animals, short duration of the trial, loss of infected quarters in the experimental groups, possibility of previous mammary gland damage prior to antibiotic infusion and unintentional bias in pedigree could have contributed to the lack of differences in milk production between groups. Future research should be extended in time to further investigate seasonal effects, and increase the number of control and treatment animals.

Table 5.1. Distribution of heifers calving by season

Winter (Dec 21-Mar. 20)	Spring (Mar. 21-Jun. 20)	Summer (Jun. 21- Sept. 20)	Fall (Sept. 21-Dec. 20)
11	5	26	3

5.3 Somatic Cell Count

Somatic cell count (SCC) was expected to decrease in this trial as was shown in previous research with primiparous heifers. Somatic cell count (SCC) was converted to logarithmic somatic cell score (SCS). Somatic cell score was measured between treatment groups at 14 days before calving (C-14), 14 days after calving (C+14), the first 3 months of lactation, and monthly through the first 200 days after calving using the first 10 DHIA records of each animal. SCS measured at 14 days before and 14 days after calving were taken for individual quarters while all other samples were a composite of all four quarters. Somatic cell count score (SCS) was lower in treated heifers than in control heifers throughout all stages of lactation, except for C-14.

SCC in heifers at C-14 did not differ between groups (Table 4.7). However, there was a trend towards the control group having lower SCS. It was assumed that SCS would be similar at the beginning of the study. The fact that the control heifers had lower cell counts before treatment, but decreased only by 24%, compared to 79% in treated heifers, illustrates the potential of prepartum intramammary antibiotics. It is documented in the literature that cows with lower somatic cell counts yield more milk (67). While no significant differences in milk production were apparent, the differences in cell count indicate a potential for increased milk production from heifers treated with intramammary antibiotics. This would

concur with the work of Oliver, et al. (53) which found that heifers treated with lactating cow antibiotics produced 531 kg more milk per 305 day lactation than their control counterparts.

When DHIA SCS records were analyzed for the first 3 months of lactation, the treated heifers were consistently lower than control heifers during all 3 months; however differences were not significant at any month of lactation. A trend was apparent at Month 1 of lactation towards lower SCS in treated heifers, but disappeared at Months 2 and 3. SCS was 23.6, 16.8 and 14.2% lower in treated heifers at Months 1, 2 and 3 respectively. At first glance it appears the differences in SCS during the first 3 months of lactation are not strong. However, when the first three months of lactation for each heifer was averaged and then analyzed, the difference between experimental groups was almost significant, and there was evidence of a trend at P=.07. The 200 day monthly SCS was analyzed the same way, and it displayed a trend for lower SCS (P=.11). Given the variation in DHIA testing, averaging provides a closer estimate of actual SCS. Averaging will dilute the effect of an abnormally high score caused by incorrect labeling, misreading a label, or a nervous heifer failing to let her milk down on test day. Conversely, if one was looking at the behavior of SCS curves throughout the 200 day period, averaging could destroy any meaningful data. We included data for both types of analysis of SCS where applicable.

5.4 Reproduction

Differences in reproductive performance between experimental groups were measured using services per conception, days open, days to first breeding and first service conception rate as indices of reproductive efficiency. Additionally, differences in these indices were assessed between heifers with *Staphylococcus aureus* infections and heifers without *S. aureus* infections.

Although control heifers required almost one additional breeding before pregnancy (3.71 breedings/ conception) compared to treatment heifers (2.74 breedings/conception), differences were not significant (Table 4.11). University of Tennessee researchers examined reproductive performance of cows (n=205) that had clinical mastitis. Cows with clinical mastitis required 2.9 breedings/ conception compared to 1.7 breedings in cows with no clinical mastitis (2). This difference was significant (P=.01). In our study, days open, days to first breeding, and first service conception rate did not differ between experimental groups.

The differences between heifers with *S. aureus* intramammary infections and those without were perplexing. One would expect that heifers with *S. aureus*, a major mastitis pathogen, would have decreased reproductive performance. Yet, heifers without *S. aureus* infections required 3.57 breedings per conception while heifers with *S. aureus* infections required 2.62 breedings per conception. Furthermore, heifers without *S. aureus* infections had 146 days open while heifers with *S. aureus* had 113 days open. While this data was not significant, it was interesting nonetheless. However, the likelihood of skewness caused by a unbalanced experimental population is high.

There is a scientific basis to indicate differences in reproductive performance between heifers with mastitis and heifers without mastitis (2, 24). Hockett, et al. (24) theorized that clinical mastitis may cause a change in prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) release. Furthermore, they determined that if concentrations of $PGF_{2\alpha}$ were elevated, a decrease in embryonic development and quality in addition to luteal regression may occur. While that study was done on cows induced with experimental intramammary infections, it begins to explain possible effects of mastitis on reproduction at the cellular level. Further research is

warranted to determine the full relationship between clinical and sub-clinical mastitis caused by gram- positive pathogens and decreased reproductive performance.

5.5 Ketones

The metabolic effect of clinical or sub-clinical mastitis in cattle has received little attention. First calf heifers generally do not show signs of clinical ketosis and research to determine the effects of ketosis on the immune system in vitro have been conflicting (16, 29). Ketone levels were measured between treated and control heifers at 7 and 14 days postpartum. There were no differences or trends apparent between experimental groups at either time. Additionally, ketone concentrations between heifers with *S. aureus* intramammary infections and heifers without *S. aureus* were evaluated. There were no statistical differences or trends, however control heifers consistently had higher ketone concentrations throughout both weeks when averaged. As seen in Tables 4.12 and 4.13, there were no differences or trends between the treatment groups.

Lactating cows normally have low concentrations of circulating ketone bodies due to the metabolic demand of lactation. When glucose demand exceeds the gluconeogenic capacity of the liver, clinical ketosis develops as ketone concentrations increase in the system. Risk factors for ketosis include herd, parity, genetics, season and body condition (31). Higher milk ketone levels were observed at week 2. Previous studies showed that peak incidence of ketones occurred at weeks 2 and 3 postpartum (20, 31). However, at week 1 10-30% of cows had subclinical ketosis. This data indicates that measurement of ketone concentrations may have been more useful if done at week 3 post-calving. Milk samples were obtained at the AM (0200 h) or PM (1300 h) milking for determination of ketones . Research indicates additional variables that may skew milk ketone levels as measured here

(20). These include high milk SCC, feeding of poor silage and diurnal variation. Perhaps future studies should extract milk samples at a uniform time of day. Furthermore, a more detailed analysis of milk ketones in cows with high SCC may have been productive. We examined the relation between cows with *S. aureus* infections and concentration of milk ketones. Given that cows with *S. aureus* display high SCS, the somewhat higher levels of ketone bodies present in these animals present a problem. Were the slightly higher ketones in *S. aureus* heifers really elevated or were they due to increased SCC? The low experimental numbers in our study prevent us from making any conclusions. Control heifers had significantly higher SCS than treatment heifers at week 2 and during the first month of lactation, when the ketone samples were taken. Future studies should examine this problem and provide means for measuring physiological ketone levels other than or in conjunction with milk ketone levels.

Given the relation between negative energy balance and poor reproductive performance, perhaps an analysis of heifers with high ketone concentrations and their subsequent reproductive performance would be beneficial. Results from this study could warrant further studies examining the relationship between sub-clinical mastitis and overall metabolism.

CHAPTER 6. SUMMARY

There were three objective of this study: One was to determine the effect of a lactating cow cephapirin product administered two weeks prepartum on existing intramammary infections in primigravid dairy heifers. Second, to determine the effect of a lactating cow cephapirin product administered two weeks prepartum on milk production and somatic cel count in primiparous dairy heifers. Third was to evaluate the effect of a lactating cow cephapirin product administered two weeks prepartum on reproductive performance and metabolic status in primiparous heifers.

Forty six primigravid Jersey heifers from the Hill Farm mastitis research laboratory dairy were randomly assigned to treated or control groups. Animals with even numbered ear tags (n=21) were assigned to the treated group while animals with odd numbered ear tags were assigned to the control group (n=25). Treated animals received an intramammary infusion of 200mg cephapirin sodium in each quarter (Fort Dodge Animal Health, Fort Dodge, IA) 14 days prior to expected date of parturition. Control animals received no infusion. The study was initiated in January 2002 and completed in April 2003. Criteria examined included cure rates, milk production, somatic cell count, milk ketone concentration and reproductive performance. Additionally, seasonal effects on milk persistency and ketones were examined, as well as the effect of *S. aureus* intramammary infections on ketone levels and reproductive performance.

The intramammary infusion of lactating cow antibiotics 14 days prepartum decreased the number of infected quarters by 78% in treated group. Spontaneous cure rate in control heifers was 21%. Cure rate of quarters with *S. aureus* in treated heifers was 80%, while the control heifers had a spontaneous recovery rate of 23%.

There were statistical differences in milk production between groups during the study. Control heifers consistently produced more milk throughout the entire lactation than treated heifers (P=.0001). Production at other times was also higher in the control heifers, but not significantly different. Reasons for unexpected production by the control group may include genetic differences and season of calving. When persistency of milk production was measured, control heifers were more persistent when calving during cool weather as compared to warm weather (P=.05). Treated heifers had a trend towards increased persistency when calving during cool weather (P=.13).

Intramammary treatment lowered somatic cell count score in treated heifers as compared to control heifers at day C+14 (P=.005). Treated heifers had a tendency for lower SCC than control heifers during the first 3 months of lactation (P=.07) and the first 200 days of lactation (P=.11).

There were no significant differences in reproductive performance between groups. Control heifers required more services per conception (3.71) than treated heifers (2.74) but the difference was not significant (.33). Heifers with *S. aureus* infections required fewer breedings per conception (2.62) than heifers without *S. aureus* infection (3.57) however the difference was not significant (P=.29). Also, heifers with *S. aureus* infections had fewer days open (n=113) than heifers without *S. aureus* (n=146) but the difference was not significant (P=.30).

Milk ketone levels were measured at weeks 1 and 2 postpartum. There were no differences in the concentration of milk ketones between groups at either time. Additionally, there were no differences in milk ketones between heifers with *S. aureus* infections and those

without. However, heifers with *S. aureus* infections consistently had higher milk ketone levels than those without.

In conclusion, some useful information was confirmed by this study. Prepartum intramammary antibiotics will significantly improve cure rates in primiparous heifers. It also trended to lower somatic cell count scores. There was a tendency for treatment to have an effect on reproduction and ketone levels, but this was not strong enough to draw conclusions. Additional research is still needed to determine effects of prepartum, intramammary treatment on milk production, reproductive performance, and ketone concentration.

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VITA

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He attended Brunswick area schools and was active in the Boy Scouts of America, earning his Eagle Scout award in 1993. He graduated from the University of Maine in 1998 with a baccalaureate degree in animal science.

The writer has been fortunate to have held unique positions at various dairy institutions. These include President of the first University of Maine student run dairy farm, and a summer internship which resulted in employment and nutritional research experience at the W.H. Miner Agricultural Institute in Chazy, New York. The Miner Institute is where he originally developed interest in mastitis and lactation physiology.

Additionally, he has worked for Dirigo Holsteins, a dairy farm in Auburn, Maine, that is active in the world of embryo transfer and Holstein genetics. At Louisiana State University he was a research herd supervisor for the LSU teaching and research dairy farm in Baton Rouge, Louisiana, from January 2000 through July 2001 and herd manager for the LSU AgCenter Hill Farm Mastitis Research Laboratory dairy from April 2002 through August 2003.

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